On the Occurrence of Encystation in Trypanosoma grayi *Novy, with Remarks on the Method of Infection in Trypanosomes generally.*

By E. A. Minchin, M.A.

(Communicated by Professor E. Ray Lankester, F.R.S. Received November 3,—Read November 8, 1906.)

In a tsetse-fly dissected and examined by me in the laboratory of the Sleeping Sickness Commission at Entebbe, Uganda, it was found that not only was the intestine swarming with *Trypanosoma grayi*, but the proctodæum also contained vast numbers of trypanosomes. Under moderate magnification they could be seen in dense clumps attached to the wall of the proctodæum, each clump having a superficial resemblance to a patch of mould, the whole mass, however, vibrating with the movements of the flagella. It was rather uncommon to find these parasites in the hind gut, and I at once made smears from different regions of the digestive tract, and carefully preserved and stained them. As it was but a short time before my departure from Entebbe, I was not able there to do more than glance at my preparations, but I noticed at once an important fact, which since my return to England I have been able to confirm and extend by careful study of my slides: namely, that in the hind gut the trypanosomes are in process of becoming encysted. Before proceeding to describe the encystment, I will say a few words about the conditions of the occurrence of these trypanosomes, and the manner in which they were preserved.

The tsetse-fly in question was one of a batch caught by our fly-boys at Entebbe on November 2, and fed the next day on a monkey infected with *Trypanosoma gambiense* from the cerebro-spinal fluid of a Sleeping Sickness patient. On every subsequent day these flies were fed on a healthy guinea-pig, and a certain number of flies were dissected daily and examined, until the batch was used up. Without going into details, it is sufficient to say that *T. gambiense* was found sparingly in the flies dissected up to 96 hours, that is to say, on November 4, 6, and 7. After this date *T. gambiense* disappeared completely and could not be found in any of the flies dissected. The fly in which the encystment was discovered was dissected on November 14. It must, of course, have been infected with *T. grayi* when caught, and, as I have stated, it had been kept 11 days in the laboratory and fed daily. At the autopsy it was found to be gorged with blood, and with all its organs perfectly normal and healthy in appearance. I examined the salivary glands and genital organs (testes and vesiculæ seminales) without finding

anything resembling a trypanosome. Finally the digestive tract was examined, with the results stated. No trypanosomes were found in the proventriculus.

The films were made in the usual way by drying the smears, which were then fixed with methyl alcohol and stained with Giemsa's stain. A few were kept unfixed and have been fixed and stained for me recently by my assistant, Dr. J. D. Thomson, with excellent results. It is a matter of great regret to me now that none of my smears of this fly were fixed by the osmic-vapour method. The method of drying smears is a drastic one, which is likely to deform finer details, and in the present instance the cyst-wall is often injured, being evidently of a soft consistence.

The gut was divided into four regions and smears made from each. In the anterior region of the intestine the ingested blood is of red colour, very thick, and jelly-like and difficult to smear out nicely. Further back it gets more fluid and begins to turn black. In the hinder part of the intestine the blood is black and fluid. I refer to these three regions briefly as the red, red-black, and black blood respectively. The black blood stops sharply and suddenly at the point at which the Malpighian tubules enter the gut. The proctodæum contains no blood, but only a yellowish fluid containing innumerable coarse granules.

I proceed now to describe briefly the process of encystation observed by me in the fly. A glance at the preparation shows many different stages of the process side by side. In the first place we find individuals in which encystment has not begun (figs. 1, 2). These are forms for the most part very slender and smaller than any of the forms of *T. grayi* found in the intestine of this or other tsetse-flies; but their most striking feature is the absence of any distinct undulating membrane, so that they bear a great resemblance to the genera *Herpetomonas* and *Crithidia*, especially the latter. The flagellum is long and appears to run down the side of the body. The blepharoplast has the large size and rod-like form characteristic of *T. grayi*. The nucleus is either compact or broken up into granules of chromatin. Division stages have been found, but are very rare.

In the first stages of encystment the flagellum becomes shortened and stains more deeply, suggesting that as it diminishes in length it becomes thickened. At the same time the cyst begins to appear as a layer of substance which stains reddish with Giemsa, forming a cap at the hinder pole (figs. 3, 4). These two processes continue until on the one hand the flagellum is completely retracted and on the other hand the body is enveloped in a pear-shaped cyst, at first incomplete towards the pointed end (figs. 5, 6). The flagellum appears to become retracted into a pink-staining vacuole (fig. 5,

fl. v.), which reminds one of the flagellar vacuole described by Leishman during the formation of a flagellum in the Leishman-Donovan bodies in cultures, but here the sequence of events is inverted, as it were. Finally the pink vacuole disappears, but a streak, which gradually fades away, can be seen for a time in front of the blepharoplast (fig. 6). The cyst closes up round the pointed end of the body, and then changes in form, becoming first oval (fig. 7) and then irregularly circular in outline (fig. 8). In the circular cysts, which are the last stage, it is difficult to make out much detail, but in favourable examples they can be seen to contain hyaline protoplasm, staining faintly bluish, with the dark purple-stained blepharoplast and the nucleus generally represented by irregularly scattered granules, staining a purplish-red tint. The cyst-wall also stains strongly and appears slightly redder than the nucleus.

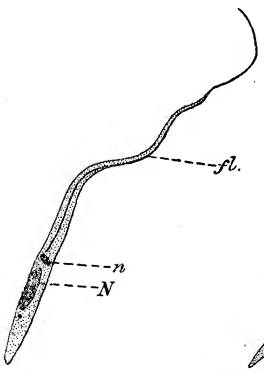

Fig. 1.

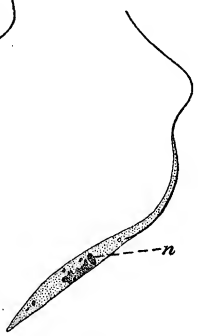

Fig. 2.

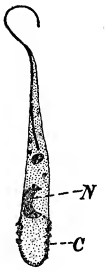

Fig. 3.

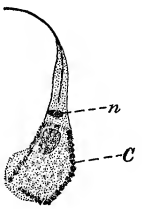

Fig. 4.

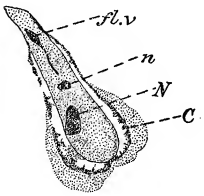

Fig. 5.

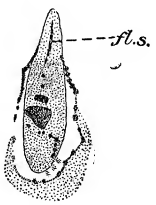

Fig. 6.

Fig. 7.

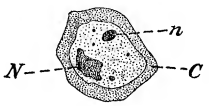

Fig. 8.

I will now state briefly the conditions observed in other parts of the gut of the fly in which the cysts were found. The red blood was found teeming with trypanosomes, nearly all large forms of indifferent type. Very few young forms were found, and male forms were very scarce. No pronounced female types were found in this region, though many of the large indifferent forms approach the female type in the shortness of their free flagellum. In

the red-black blood the conditions were similar, but young forms are becoming commoner. In the black blood young forms predominate, and large individuals are comparatively infrequent. Thus in the gut of this fly as a whole we find great rarity of differentiated sexual forms, but a swarm of indifferent forms which in the hinder part of the intestine give rise to very numerous young forms, and these in their turn would appear to pass into the small, *Herpetomonas*-like forms found in the proctodæum, starved-looking creatures which, in a medium where there is probably no nutriment, go through the process of encystment already described.

Having so far confined myself strictly to matters of fact, I will now offer a few suggestions and speculations as to the probable significance of the encystation in the economy of the life-cycle of the parasite. In the first place, the resemblance of these cysts, and especially the pear-shaped forms, to the "Schleim-Cysten" described by Prowazek* in *Herpetomonas muscæ-domesticæ*, Burnett, is very marked, and has struck everyone to whom I have shown both my preparations and Prowazek's figure. I think there can be no doubt that they are similar bodies, and have a similar function, that is to say, that they are destined to pass out of the gut of the fly with its dejecta. Very numerous analogies in support of this inference could be cited from other parasitic Protozoa. The question which interests us most is: what becomes of them after being cast out from the fly? In the absence of any observations or experiments upon this point, one can only draw conclusions from the analogy of what is known in other cases. In the case of *H. muscæ-domesticæ* the cysts are scattered about everywhere, and, as house-flies are far from being particular in their feeding or sanitary in their habits, a fly runs every chance of infecting itself with the *Herpetomonas* by swallowing with its food cysts dropped by another fly. There can be no doubt whatever, it seems to me, that this is the manner in which the house-fly acquires the infection of the *Herpetomonas*. The case is, however, very different with the tsetse-fly, which does not haunt the enclosed spaces and insanitary surroundings of the house-fly, but lives a free, open-air life by lake and forest. Nor is the tsetse-fly a foul feeder like the house-fly. My assistant, Mr. Degen, and myself made many attempts to feed tsetse-flies on all kinds of food, but always without the least success. On the other hand, the tsetse-fly is a greedy blood-sucker, and will attack anything from a frog, lizard, or bird to a hippopotamus, but, in my opinion, it does not feed in any other way. Further, the tsetse-fly is not like the *Stomoxys*, which lays its eggs in dung; it is viviparous, as is well known, and nourishes its larva in the uterus until full-grown.

* 'Arbeiten a. d. k. Gesundheitsamte,' Berlin, vol. 20 (1904), p. 446.

For all these reasons it seems to me in the highest degree improbable, indeed I may say impossible, that a tsetse-fly would ever infect itself by sucking up cysts dropped by another fly, or that a parasite which had to depend on this method of dissemination could maintain its existence in the tsetse-fly. The only possible destiny I can imagine for these cysts is to be swallowed accidentally by some vertebrate, the (as yet unknown) host of *Trypanosoma grayi*, in order to germinate in its digestive tract, to pass thence into the blood, and to be taken up again with the blood by the tsetse-fly. A cycle of this type is as yet unknown, but there are abundant analogies for all parts of it. In the first place, it is a common thing for animals to have protozoan parasites in the gut, which they take up in the encysted condition after they have been dropped by another individual. Without multiplying instances unnecessarily, I may point out that Schaudinn proved the infection of *Amœba coli* to originate in this way, and that it is a common human parasite in regions where sanitation has not advanced beyond the primitive condition of *épandage par terre*. In the second place, there are many instances among Sporozoa of cysts germinating in the intestine and liberating motile forms which then pass through the wall of the gut into other organs of the body.

In a former communication by my colleagues,* Lieutenants Gray and Tulloch, and myself, we were able to confirm Bruce's results as to the existence of direct mechanical infection by means of the tsetse-fly, which, if it stabs its proboscis first into an infected animal and then soon after into a healthy one, can infect the latter. We were not able to demonstrate, however, what I may term cyclical infection, which at present has not been shown to exist. I suggest that there are two possible modes of cyclical infection, in the dissemination of protozoan blood-parasites by biting insects generally. In one method, which I may term *inoculative*, the parasite, after going through developmental changes in the insect, passes back again into a second vertebrate host through the proboscis, as in the case of malaria transmitted by a mosquito. In the other method, which I propose to term *contaminative*, the parasite taken up by the biting insect, after going through developmental changes within its gut, would pass out of it through the anus, and infect the vertebrate host by contaminating its food or drink. We have all of us (I speak for myself) been imbued hitherto with the idea that the cycle of the trypanosome in the tsetse-fly must be of the inoculative type, and have failed to find it. I wish to suggest strongly to those working on the subject of trypanosome-infection the desirability of making experiments and observations to prove or disprove the existence, in the insect which

* 'Roy. Soc. Proc.,' B, vol. 78 (1906), p. 242.

disseminates the parasite, of a life-cycle which results in a contaminative infection of the vertebrate host.

A full account of the trypanosome-cysts described in this note, and of other points relating to *Trypanosoma grayi*, will be published, with illustrations, in the 'Quarterly Journal of Microscopical Science.'

DESCRIPTION OF THE FIGURES.

FIGS. 1 and 2.—Free-swimming *Herpetomonas*-like forms of *Trypanosoma grayi* from the proctodæum of the tsetse-fly, before encystation has commenced; *N.*, principal nucleus; *n.*, blepharoplast; *fl.*, flagellum. ×2000.

FIG. 3.—Commencement of encystation. The flagellum is becoming retracted, and the first appearance of the cyst-secretion is seen at *C*. ×2000.

FIG. 4.—Similar stage, slightly more advanced. The cyst-wall (*C*) is damaged at one point. ×2000.

FIG. 5.—Flagellum completely retracted, represented by a vacuole (*fl. v.*). ×2000.

FIG. 6.—The flagellar vacuole is absorbed and the flagellum is represented only by a delicate streak (*fl. s.*). Cyst much damaged. ×2000.

FIG. 7.—The cyst-secretion now extends all round the body. ×2000.

FIG. 8.—Rounded-off cyst, the final stage. Letters as before. ×2000.